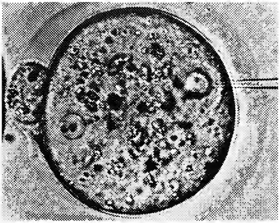


Figure 4.19
Insertion of new DNA into embryonic cells. Here, DNA (from cloned genes) is injected into the pronucleus of a mouse egg. (From Wagner et al. 1981; photograph courtesy of T. E. Wagner.)

jected into a *Drosophila* oocyte, it can integrate itself into the embryo's DNA, providing the organism with the new gene (Spradling and Rubin 1982).

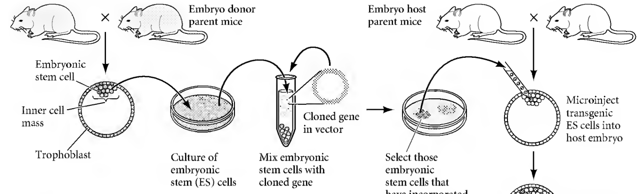
CHIMERIC MICE. The techniques described above have been used to transfer genes into every cell of the mouse embryo (Figure 4.20). During early mouse development, there is a stage (the blastocyst) when only two cell types are present: the outer trophoblast cells, which will form the fetal portion of the placenta, and the inner cell mass, whose cells will give rise to the embryo itself. The inner cells are the cells whose separation can lead to twins (see Chapters 3 and 11), and if an inner cell mass blastomere of one mouse is transferred into the embryo of a second mouse, that donor cell can contribute to every organ of the host embryo.

Inner cell mass blastomeres can be isolated from an embryo and cultured in vitro; such cultured cells are called **embryonic stem cells (ES cells)**. ES cells are almost totipotent, since each of them can contribute to all tissues except the trophoblast if injected into a host embryo (Gardner 1968; Moustafa and Brinster 1972). Moreover, once in culture, these cells can be treated as described in the preceding section so that they will incorporate new DNA. This added gene (the transgene) can come from any eukaryotic source. A treated ES cell (the entire cell, not just the DNA) can then be injected into another early-stage mouse embryo, and will integrate into this host. The result is a **chimeric mouse.*** Some of the chimera's cells will be derived from the host's own embryonic stem cells, but some portion of its cells will be derived from the treated embryonic stem cell. If the treated cells become part of the germ line of the mouse, some of its gametes will be derived from the donor cell. If such a chimeric mouse is mated with a wild-type mouse, some of its progeny will carry one copy of the inserted gene. When these heterozygous progeny are mated to one another, about 25% of the resulting offspring will carry two copies of the inserted gene in every cell of their bodies (Gossler et al. 1986). Thus, in three generations—the chimeric mouse, the heterozygous mouse, and the homozygous mouse—a gene cloned from some other organism will be present in both copies of the chromosomes within the mouse genome. Strains of such transgenic mice have been particularly useful in determining how genes are regulated during development.

GENE TARGETING ("KNOCKOUT") EXPERIMENTS. The analysis of early mammalian embryos has long been hindered by our inability to breed and select animals with mutations that affect early embryonic development. This block has been circumvented by the techniques of gene targeting (or, as it is sometimes called, gene knockout). These techniques are similar to those that generate transgenic mice, but instead of adding genes, gene targeting *replaces* wild-type alleles with mutant ones. As an example, we will look at the gene knockout of bone morphogenetic protein 7 (BMP7). Bone morphogenetic proteins are involved in numerous developmental interactions whereby one set of cells interacts with other neighboring cells to alter their properties. BMP7 has been implicated as a protein that prevents cell death and promotes cell division in several developing organs.

Dudley and his colleagues (1995) used gene targeting to find the function of *Bmp7* in the development of the mouse. First, they isolated the *Bmp7* gene, cut it at one site with a restriction enzyme, and inserted a bacterial gene for neomycin resistance into that site (Figure 4.21). In other words, they mutated the *Bmp7* gene by inserting into it a large piece of foreign DNA, destroying the ability of the BMP7 protein to function. These mutant *Bmp7* genes were electroporated into ES cells that were sensitive to neomycin. Once inside the nucleus of an ES cell, the mutated *Bmp7* gene may replace a normal allele of *Bmp7* by a process called homologous recombination. In this process, the enzymes involved in DNA repair and replication incorporate the mutant gene in the place of the normal copy. It's a rare event, but such cells can be selected by growing the ES cells in neomycin. Most of the cells are killed by the drug, but the ones that have acquired resistance from the incorporated gene survive. The resulting cells have one normal *Bmp7* gene and one mutated *Bmp7* gene. These heterozygous ES cells were then microinjected into mouse blastocysts, where they were integrated into the cells of the embryo. The resulting mice were chimeras composed of wild-type cells from the host embryo and heterozygous *Bmp7*-containing cells from the donor ES cells. The chimeras

*It is critical to note the difference between a chimera and a hybrid. A **hybrid** results from the union of two different genomes within the same cell: the offspring of an *AA* genotype parent and an *aa* genotype parent is an *Aa* hybrid. A **chimera** results when cells of different genetic constitutions appear in the same organism. The term is apt: it refers to a mythical beast with a lion's head, a goat's body, and a serpent's tail.



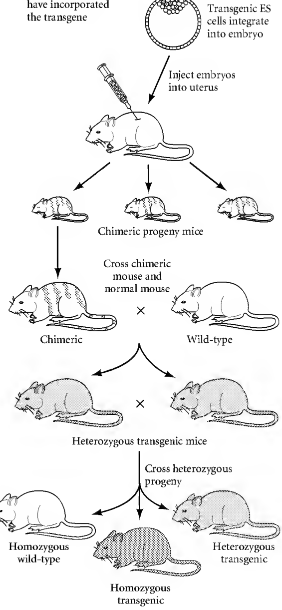


Figure 4.20
Production of transgenic mice. Embryonic stem cells from a mouse are cultured and their genome altered by the addition of a cloned gene. These transgenic cells are selected and then injected into the early stages of a host mouse embryo. Here, the transgenic embryonic stem cells integrate with the host's embryonic stem cells. The embryo is placed into the uterus of a pregnant mouse, where it develops into a chimeric mouse. The chimeric mouse is then crossed with a wild-type mouse. If the donor stem cells have contributed to the germ line, some of the progeny will be heterozygous for the added allele. By mating heterozygotes, a strain of transgenic mice can be generated that is homozygous for the added allele. The added gene (the transgene) can be from any eukaryotic source.

were mated to wild-type mice, producing progeny that were heterozygous for the *Bmp7* gene. These heterozygous mice were then bred with each other, and about 25% of their progeny carried two copies of the mutated BMP7 gene. These homozygous mutant mice lacked eyes and kidneys (Figure 4.22). In the absence of BMP7, it appears that many of the cells that normally form these two organs stop dividing and die. In this way, gene targeting can be used to analyze the roles of particular genes during mammalian development.

WEBSITE 4.8 **Knocking out specific genes at specific times and places.** Some genes are active early in development and also later in development. If their early function is critical to the life of the embryo, one cannot find out what the later function is by knocking out the gene. So techniques have been developed to knock out a gene only in particular cell types.

Determining the function of a message: Antisense RNA

Another method for determining the function of a gene during development is to use "antisense" copies of its message to block the function of that message. Antisense RNA allows developmental biologists to analyze the action of genes that would otherwise be inaccessible for genetic analysis.

Antisense messages can be generated by inserting cloned DNA into vectors that have promoters at both ends of the inserted gene. When the vector is incubated with nucleotide